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The molecular epidemiology of respiratory viruses in military trainees in Iran

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Abstract

Background: Military populations are more prone to respiratory infections worldwide. There is a dearth of research about the role of viral pathogens in the etiology of respiratory infections in military trainees in Iran. Hence, we aimed to investigate the molecular epidemiology and clinical symptoms of respiratory viruses among this population.

Methods: This cross-sectional study was performed on 400 military trainees with symptoms of respiratory infection, referred to the military medical clinic center in the basic military training camp of the General Staff of the Armed Forces of the Islamic Republic of Iran. Nucleic acid extraction from the throat or nasopharyngeal swab samples was performed by an automated extraction system. The extracts were then analyzed by the CLART® PneumoVir array system for the detection of respiratory viruses.

Results: All military trainees were male, aged between 18 and 57 years (mean: 21.69 years). Sore throat (75.5%), rhinorrhea (63.2%), cough (59.2%), fever (59.2%), and nasal congestion (50.5%) were amongst the most common symptoms. Overall, viral pathogens were detected in a total count of 124 (31%). The most commonly detected viruses were rhinovirus (7.2%), respiratory syncytial virus A (7.2%) and influenza B virus (6%).

Conclusion: This study was an important first step for understanding the etiological role of viral pathogens in respiratory infection among military trainees population in Iran. Our results indicated that rhinovirus, respiratory syncytial virus A and influenza B virus are important viral pathogens causing respiratory infection in military trainees, respectively. However, further multi-center studies with larger sample size are strongly recommended to confirm our findings.

Keywords: Military trainees, Respiratory infection, Microarray, Iran, Viral infection

Conflicts of Interest: None declared

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Introduction

The role of respiratory infections as key causes of morbidity and mortality among military trainees is well-

recognized. Compared to the young healthy adults' population, military trainees undergoing basic military training

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↑What is "already known" in this topic:

Some previous studies reported that adenoviruses, influenza viruses, human rhinoviruses, and coronaviruses are the predominant viruses detected in the military population. However, there is no report on the prevalence of respiratory viruses in the military population in Iran and even in the Middle East.

→What this article adds:

Our findings have shown that 31% of all military trainees with respiratory infection were associated with different viral agents. Human rhinovirus (7.2%), human respiratory syncytial virus A (7.2%), and influenza B virus (6%) were the most frequently detected respiratory viruses in our study, followed by bocavirus (3%), influenza A H1N1 (2.2%), influenza A H3N2 (1.5%), human respiratory syncytial virus B (1.5%), adenovirus (1.5%), and human coronavirus 229E (0.7%).

course appear to be at increased risk of acquiring and transmitting respiratory infections (1). Every year, a large number of cases of respiratory infections occur in basic military trainees worldwide which the majority of them are resulting from viral infections. Respiratory infections have a tremendous impact on the military population and are responsible for 25–30% of hospitalization in the U.S. military (2). Affected trainees generally require a sufficient period of recovery which can lead to a longer training duration and a significant higher rate of viral transmission to newer cohorts (3). The higher vulnerability to respiratory disease epidemics observed in military trainees has been attributed to several causes including crowded habitation, demanding physical training program, stressful working environment, and insomnia (4, 5).

Numerous previous studies reported that adenoviruses, influenza A and B, human rhinoviruses, and coronaviruses are the predominant viruses detected in the military population. Outbreaks of adenovirus-associated respiratory disease have been reported globally in the military environments (3, 5-8). It has been suggested that adenovirus infection is associated with male gender, as well as direct contact with an infected person with respiratory symptoms 10 days prior to the onset of illness (8). Outbreaks of influenza viruses A and B have also been associated with much morbidity and mortality, especially influenza A (H1N1) pdm09 virus infection (9-13). It has been proposed that crowded living quarters, obesity, asthma, and age group (younger than 40 years) are amongst the major risk factors for acquiring influenza infections (14). Human rhinoviruses are the most important causative agents of the common cold and associated with more complicated upper respiratory tract infections (15, 16). Approximately, all the human rhinoviruses have been detected in military trainees during respiratory infection (17). It has been also well documented that human rhinoviruses are associated with lower respiratory tract infections (18, 19). Nevertheless, there are other viruses associated with respiratory infections which are not well studied in military populations, including respiratory syncytial viruses (20-22), human bocaviruses (23-25), human parainfluenza viruses (26), metapneumoviruses (27), and echoviruses (28).

Until now, there is no study which aimed to investigate the prevalence of viral agents responsible for respiratory infections among the military population in Iran. Using micro-array technology, the objective of our study was to evaluate the molecular epidemiology of 17 viral pathogens causing respiratory infections among 400 military trainees in a large military training camp in Tehran, over a period of time from January to March 2017. The data resulted from this study can be employed to take preventive measures aimed to reduce the disease burden and prevent future outbreaks.

Methods

Study population and samples collection

Study participants were symptomatic military trainees in the basic military training camp of the General Staff of the Armed Forces of the Islamic Republic of Iran. All trainees with symptoms of respiratory tract infection including oral

temperature of $\geq 38^{\circ}\text{C}$, cough, sore throat, rhinorrhea, etc. referred to the military medical clinic center were included in this study. Exclusion criteria were a prior history of chronic respiratory disease such as asthma and chronic obstructive pulmonary disease (COPD), recent antiviral therapy, and military personnel. Those trainees meeting the case definition received a written and verbal briefing and were invited to give voluntary and informed consent to participate. This cross-sectional study was designed to enroll 400 subjects over a period of 3 months, from January to March 2017. This study has been approved by the Ethics Committee of Iran University of Medical Sciences under the Ethics Code IR.IUMS.REC1394.26051. Throat or nasopharyngeal swab specimens of patients were collected by Dacron swabs, placed in viral transport media and stored at -70°C until use.

Nucleic acid extraction

Nucleic acid (DNA/RNA) extraction was performed with 200 μl of each collected specimen using the ZP02003 MagPurix Viral Nucleic Acid Extraction Kit (Zinexts Life Science Corp, New Taipei, Taiwan) by the MagPurix 12 Series (Zinexts Life Science Corp, New Taipei, Taiwan) consists of compact bench-top robotic workstations for automated, according to the manufacturer's protocol. Total nucleic acids concentration and purity were evaluated using the Nanodrop 2000 spectrophotometer (Thermo Scientific, USA), and subsequently stored at -70°C until further analysis.

Microarray processing

CLART[®] PneumoVir array system (Genomica, Coslada, Madrid, Spain) was used to identify genomic sequences of human respiratory viruses, according to the manufacturer's recommendations. Influenza viruses (A, B, and C), human parainfluenza viruses 1-4 (subtypes A and B), respiratory syncytial virus (types A and B), human rhinovirus, metapneumovirus (subtypes A and B), echovirus, adenovirus, human coronavirus 229E, and bocavirus have been investigated by this technique.

This system was based on reverse transcription polymerase chain reaction (RT-PCR) for amplifying a specific 120–330 bp target of the viral genome. RT-PCR was performed in two different ready-to-use amplification tubes. Amplified products were labeled with biotin in the course of the process. After the amplification, biotin-labeled products were hybridized with their respective specific probes immobilized at different sites in the array. Next, the samples were incubated with the streptavidin-peroxidase to generate insoluble products which precipitate at the hybridization sites in the array. Hybridization happened in a low-density microarray containing triplicate probes specific to viruses.

Statistical methods

Statistical analysis was performed using IBM SPSS version 18 (Inc., Chicago, USA) and Microsoft Office 2013 (Excel). Descriptive analysis was carried out using percentage and simple frequencies. Data was reported as count and percentage. Clinical signs and symptoms were

counted, and the corresponding empirical proportions were calculated with 95% confidence intervals (CIs) to measure the overall symptom load.

Results

A total of 400 Iranian military trainees with clinical diagnostic criteria for respiratory infection were enrolled in the survey. All participants were male, with a mean age of 21.69 ± 4.9 years (range from 18 to 57 years). Most prevalent complaints of patients referred to the military medical clinic center were sore throat ($n=302$; 75.5%), rhinorrhea ($n=253$; 63.2%), cough ($n=237$; 59.2%), fever ($n=237$; 59.2%), and nasal congestion ($n=202$; 50.5%) (Fig. 1). Of

the 400 samples, 124 (31%) were positive for respiratory viruses. Human rhinovirus ($n=29$; 7.2%), human respiratory syncytial virus A ($n=29$; 7.2%), and influenza B virus ($n=24$; 6%) were the most frequently detected respiratory viruses in our study, followed by bocavirus ($n=12$; 3%), influenza A H1N1 ($n=9$; 2.2%), influenza A H3N2 ($n=6$; 1.5%), human respiratory syncytial virus B ($n=6$; 1.5%), adenovirus ($n=6$; 1.5%), and human coronavirus 229E ($n=3$; 0.7%). Other viruses including influenza C virus, human parainfluenza viruses, metapneumovirus, and echovirus have not been detected in any of the samples (Table 1, Fig. 2). It's worth noting that no co-infections were detected in our study. The most cases of dyspnea

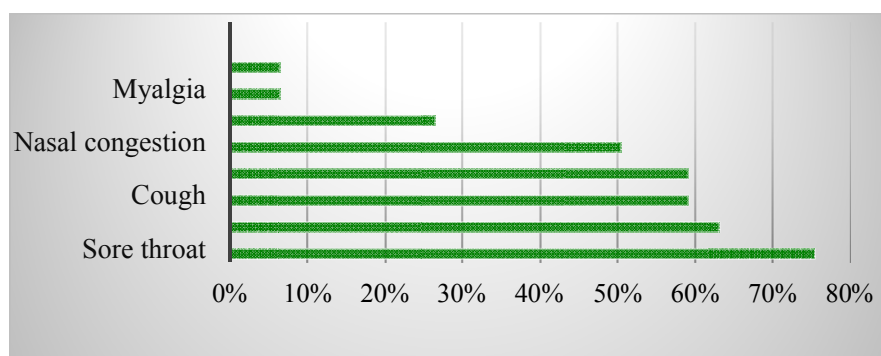


Fig. 1. Frequency distribution of clinical symptoms of respiratory infection among 400 military trainees enrolled in the study

Table 1. Viral etiology and clinical characteristics of military trainees with respiratory tract infection

Viral etiology	Number of positive cases (n)	Percent (%) with 95% confidence interval	Clinical symptoms (n)
Rhinovirus	29	7.2 (5 - 10)	Sore throat (29) Rhinorrhea (28) Nasal congestion (25) Cough (20) Headache (15) Fever (10)
Respiratory syncytial virus A	29	7.2 (5 - 10)	Cough (27) Rhinorrhea (26) Nasal congestion (25) Sore throat (22) Fever (20) Dyspnea (4)
Respiratory syncytial virus B	6	1.5 (0.6 - 3.4)	Nasal congestion (4) Sore throat (4) Fever (3) Cough (3) Rhinorrhea (3) Dyspnea (1)
Influenza B	24	6 (4 - 8.9)	Fever (21) Sore throat (20) Headache (16) Rhinorrhea (13) Cough (8) Myalgia (5) Nasal congestion (3) Dyspnea (2)
Influenza A H1N1	9	2.2 (1.1 - 4.4)	Fever (6) Sore throat (5) Cough (3) Myalgia (3) Headache (2) Rhinorrhea (2)
Influenza A H3N2	6	1.5 (0.61 - 3.4)	Nasal congestion (2) Fever (4) Sore throat (2) Cough (2) Nasal congestion (1) Rhinorrhea (1)

Table 1. Ctd

Bocavirus	12	3 (1.6 – 5.3)	Cough (9) Fever (5) Sore throat (4) Rhinorrhea (3) Nasal congestion (2)
Adenovirus	6	1.5 (0. 61 – 3.4)	Sore throat (5) Fever (4) Rhinorrhea (3) Cough (1)
Coronavirus	3	0.75 (0.19 – 2.4)	Sore throat (2) Nasal congestion (1) Dyspnea (1) Cough (1) Fever (1)

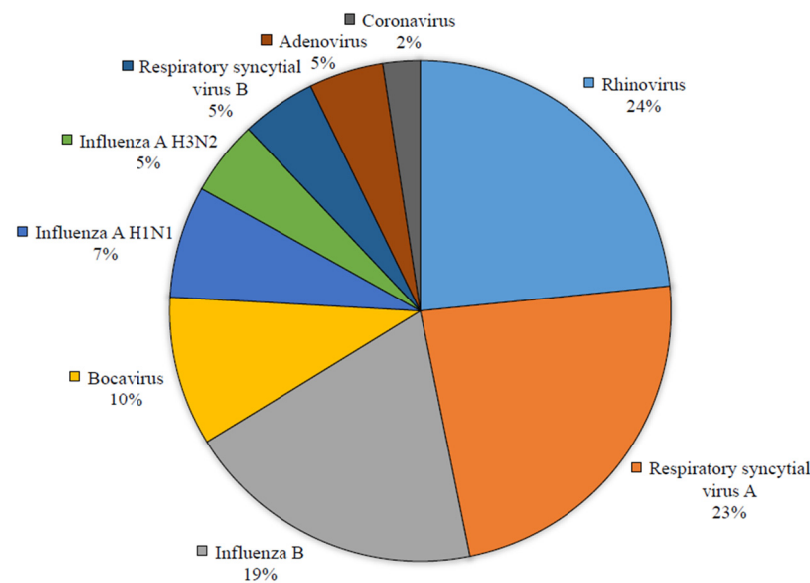


Fig. 2. Viral etiology of respiratory infection in military trainees (among 124 laboratory-confirmed cases of viruses)

(n=5; 62.5%) were in the group of patients with respiratory syncytial viruses A and B, followed by influenza B (n=2; 25%) and coronavirus 229E (n=1; 12.5%). Sore throat and rhinorrhea were the most frequent symptoms in rhinovirus infection. The most cases of myalgia were seen in influenza B (n=5; 62.5%) and influenza A H1N1 (n=3; 37.5%) infections, respectively (Table 1).

Discussion

CLART® Pneumovir array is a qualitative, highly sensitive and high-throughput technology which allows rapid detection of a broad spectrum of human respiratory viruses at a time, particularly to detect viruses that which are not culturable in the laboratory, and also those that are not detectable by the standard multiplex RT-PCR (29, 30). In the present study, CLART® Pneumovir array was used to detect 17 common respiratory viruses on 400 throat or nasopharyngeal swab specimens of Iranian military trainees which were collected during January to March 2017. Our findings have shown that 31% of all patients with respiratory infection were associated with different viral agents. According to our results, rhinovirus, respiratory syncytial virus A, and influenza B virus have been responsible for ~66% of viral respiratory infections among military trainees.

Previous studies have shown that adenoviruses, influenza A and B viruses, human coronaviruses, rhinoviruses, and at lower frequencies, respiratory syncytial viruses and human parainfluenza viruses are the most common viruses leading to respiratory infection in the military environment worldwide (1, 31). Similar to our results, respiratory syncytial virus was the main causative agent of acute febrile illness in the Republic of Korea Air Force boot camp from May to July 2011 (32). O'Shea et al. showed that among 54 British military recruits receiving basic training with respiratory symptoms, adenovirus, influenza viruses and respiratory syncytial virus were found in 35%, 19% and 14%, respectively (33). It is notable that among influenza viruses, influenza B was more associated with symptomatic infections, which was consistent with our findings. In a large study in the Singapore military population (13), viral etiology of respiratory illnesses was investigated among 7733 cases of febrile respiratory illness (FRI) using the multiplex PCR. Their results have indicated that 49% of cases were positive for viral infection and the most commonly detected viruses among FRI cases were influenza A H1N1(13%), influenza B (13%), and coxsackievirus (9%).

The low prevalence of viral infections in our study in comparison with the other parts of the world can be at-

tributed to the existence of natural immunity to respiratory viral infections. The Iranian Muslims have several organized mass gatherings such as Hajj pilgrimage and Jum'ah (Friday prayers), likely leading to transmission of respiratory viruses between people and consequently, developing of natural immunity to them. This hypothesis can be evaluated in seroepidemiological studies.

The overall prevalence of respiratory syncytial virus (types A and B) (8.7%) in our study was lower than that found in the previous works (34, 35). We believe that the rate of respiratory syncytial virus in our study could be even higher since infection with respiratory syncytial virus in a significant proportion of patients is asymptomatic. In accordance with our findings, some studies also found that although both subtypes are infectious and circulate annually, but subtype A is the main cause of respiratory syncytial virus infection which can cause more severe complications (36). Cough, rhinorrhea, nasal congestion, sore throat, and fever were most frequently symptoms of respiratory syncytial virus infection in our investigated population. It was interesting that the majority of dyspnea cases (n=5; 62.5%) was seen in respiratory syncytial virus infection in compared with other viral infections.

Our study revealed that influenza viruses (particularly influenza B) also have an important role in causing respiratory viral infection along with respiratory syncytial virus with prevalence rate of 9.7% among military trainees. Interestingly, despite the lack of adenovirus vaccination program in Iranian military health system, we reported a low rate of adenovirus (n=6; 1.5%) in military trainees. It may be explained by the fact that influenza infection tends to occur in the winter, while adenovirus infection is more likely to occur in the spring, summer, and fall seasons. In fact, seasons can be used to help the in differential diagnosis of FRI among military trainees. It should be noted that our investigation was only performed during the winter months.

There is no report on the prevalence of respiratory viruses in the military population in Iran and other countries in the Middle East. However, several studies have been performed among the general population. For example, in a cross-sectional study in pediatric patients with respiratory symptoms in Shiraz (37), adenovirus was detected in 22% of nasopharyngeal swabs samples. In another study by Barati et al., adenovirus was detected in 6.3% of nasopharyngeal secretions samples from 160 children with upper respiratory tract infection, and cervical adenopathy was the most common symptom in adenovirus infection (38). The higher prevalence of adenovirus infection in children can be explained by the fact that adenovirus shedding is more prolonged in children than the adults.

Conclusion

This study was an important first attempt to determine the molecular epidemiology and clinical symptoms of respiratory viruses circulating in Iranian military trainees. However, there are some limitations that should be noted. First, samples collection were performed completely during winter. So, the estimated prevalence rates of respiratory viruses in our study may not be representative of the

actual value, and it is required to collect more data throughout four seasons of the year. Second, samples collection were conducted only from trainees stationed within one military base. Thus, further multi-center studies with larger sample size are strongly recommended to confirm our findings.

Acknowledgments

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Conflict of Interests

The authors declare that they have no competing interests.

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